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Original Article

Cardiac autonomic changes after 40 hours of total sleep deprivation in women

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ABSTRACT

Objectives: The effect of total sleep deprivation on heart rate variability (HRV) in groups of postmenopausal women on oral hormone therapy (HT) (on-HT, $n = 10$, 64.2 (1.4) years), postmenopausal women without HT (off-HT, $n = 10$, 64.6 (1.4) years) and young women ($n = 11$, 23.1 (0.5) years) was studied using a prospective case–control setup.
Methods: Polysomnography was performed over an adaptation night, a baseline night, and a recovery night after 40 h of total sleep deprivation. Time and frequency domain and nonlinear HRV from overnight electrocardiogram recordings were compared between groups during baseline and recovery nights. Further, the changes in HRV from baseline to recovery were analysed and compared between groups. Finally, correlations of HRV to percentages of sleep stages and measures of sleep fragmentation were analysed during baseline and recovery.
Results: Young women had higher HRV than older women; the most marked difference was between young and on-HT postmenopausal women. Sleep deprivation induced a decrease in frequency domain HRV in young and in off-HT women, an increase in $\alpha 2$ in off-HT women, and an increase in mean heart rate in on-HT women. The sleep deprivation effect was mainly uncorrelated to changes in sleep parameters.
Conclusions: Acute total sleep deprivation has a deleterious effect on the autonomic nervous system in young women, but an even more pronounced effect in postmenopausal women. Hormone therapy use in late postmenopause does not give protection against these changes. These harmful effects may partly explain the increased cardiovascular morbidity and overall mortality associated with sleep loss.

1. Introduction

The heart rate of a healthy person is never stationary, but oscillates around a median. This oscillation consists of both stable frequencies (eg, respiratory sinus arrhythmia and changes induced by time of day and behavioural state) and reactions to external stimuli. These changes are measured by sampling QRS-complexes in standard computerised electrocardiogram (ECG), and measuring the changes in the time interval between successive R-peaks, called heart rate variability (HRV) [1]. Heart rate variability reflects the function of the autonomic innervation to the heart. During high sympathetic output, tachycardia and a decrease in short-term variability is observed in HRV [1]. In contrast, when vagal output dominates, a slowing of heart rate and increased respiratory sinus arrhythmia can be seen [1]. Heart rate variability can be measured using a variety of linear mathematical methods and more-complex, nonlinear methods based on Chaos theory. Many calculated indexes measure rapid beat-to-beat heart rate oscillations, marking high parasympathetic activation, while others represent slower, sympathetically modulated oscillations [1]. Many HRV markers have high intercorrelations [1–3], which imply that they have similar modulatory backgrounds. There is compelling evidence that obesity, hypercholesterolemia, high blood pressure, diabetes, and lack of regular physical activity increase the risk of cardiovascular disease

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[4]. However, decreased overall and vagally mediated HRV also increase the risk of lethal and potentially lethal cardiovascular events and even predict overall mortality in the general population [5–9].

Heart rate variability is strongly influenced by sleep and sleep stages. The awake state is associated with higher sympathetic activity than any sleep stage. In stable non-rapid eye movement (NREM) sleep, vagal influence increases and is the highest in slow wave sleep (SWS) [10–13]. In rapid eye movement (REM) sleep, the autonomic input to the heart resembles that seen in the awake state, with high fluctuations between vagal and sympathetic dominance [10–13]. In several studies, acute total sleep deprivation has been shown to increase HRV indexes that are dependent on sympathetic activity [14–17]. In one study, acute sleep deprivation of 24 h did not influence HRV [18] measured in the awake state. In two studies [19,20], increased parasympathetic tone after 24–30 h of sleep deprivation was seen, and one study [21] reported an increase in both HF and LF variability. Some recent studies with 24–60 h of continuous sleep deprivation have shown an increase in sympathetic dominance in the awake state during or after prolonged sleep deprivation [14–17]. In ECG samples gathered late in the evening or during recovery sleep after sleep deprivation, a decrease in sympathovagal balance has been observed [22,23]. However, the studies supporting these observations are mostly conducted in young subjects, ignoring the effect of age. Furthermore, while women have been neglected on this study field, there are no studies addressing the effects of menopause and hormone therapy (HT) on HRV after sleep deprivation.

The previous literature has produced both pros and cons about the effect of HT on cardiovascular health [24–29]. The beneficial findings in 1990s were strongly challenged by large studies such as the Heart and Estrogen/Progestin Replacement Study (HERS) and the Women's Health Initiative (WHI) [26–28]. Further studies have established the importance of the time of the initiation of the therapy (less than 10 years after menopause), as well as the effect of dose, treatment route (oral versus transdermal), component (unopposed oestrogen versus combined oestrogen–progestagen) and the type of progestagen [29–36]. Hormone therapy has been considered to be beneficial by: dilating arteries, reducing cholesterol levels, angiotensin-converting enzyme activation, insulin resistance, and decreasing markers of inflammation [29]. In younger perimenopausal or recently postmenopausal women, HT also increases vagal tone [37–42]. However, in older postmenopausal women, either no effect [43,44] or even a decrease in vagal tone and nonlinear complexity of heart rate has been observed [45–47]. This suggests that HT may promote cardiovascular risk in older postmenopausal women [45,46]. Contrary to the overall cardiovascular effects of HT, these studies show no clear connection between its effects on HRV and the application route, the addition of progestogens or the dose of oestrogen used.

Based on the above rationale, it was hypothesised that acute total sleep deprivation in women should result in increased sympathetic dominance and decreased vagal input to the heart. Further, the present study set out to observe whether there would be an age-related decline in HRV responses to sleep deprivation and whether postmenopausal HT would modulate these responses. Therefore, the present study examined the changes between whole-night HRV measurements before and after 40 h of total sleep deprivation in a group of young women and a group of postmenopausal women both on and off HT.

2. Methods

The present study was performed as a cooperative undertaking between the sleep research units of the University of Turku, Finland and the University of Helsinki, Finland. Twenty postmenopausal women were recruited from the Turku area through announcements in the local newspapers, and 11 young women were recruited from Helsinki by an announcement at the university for a larger study exploring the effects of ageing and HT on sleep and cognition.

Two groups of postmenopausal women were studied: (1) an on-HT group [$n = 10$, mean age 64.2 (1.4) years], who used oral, continuous combined, estradiol hemihydrate 2 mg + norethisterone acetate 1 mg (Kliogest, Novo Nordisk, Bagsværd, Denmark); and (2) an off-HT group [$n = 10$, 64.6 (1.4) years]. In the off-HT group, seven women had previously used HT with the minimum time of discontinuance of 24 months. The young women [$n = 11$, 23.1 (0.5) years], using oral contraceptives [ethinyl estradiol 20 μg + desogestrel 0.15 mg (Mercilon, Organon, Oss, The Netherlands)] served as controls, and they were examined during the first days of their menstrual cycle. Exclusion criteria consisted of previous cardiovascular (hypertension controlled with medication was accepted), pulmonary, neurological, endocrinological or mental disease, and specific sleep disorders such as sleep apnoea, narcolepsy or restless legs syndrome. Women suffering from other conditions that could possibly affect sleep, like fibromyalgia and anaemia, were excluded. Other exclusion criteria included the use of medication that affects the central nervous system (CNS), alcohol abuse, smoking, and excessive caffeine intake. Before the study, blood haemoglobin, leucocytes, thrombocytes and serum thyrotropin levels were measured to ensure that they fell within normal ranges.

Subjects were first informed about the study protocol and screened for exclusion criteria in a 10–30 min telephone interview (P.P-K.). Those meeting inclusion criteria were invited for a personal interview (duration 1–1.5 h, P.P-K.) in which exclusion criteria were re-screened, a clinical interview and physical examination were made, study information was provided, and informed written consent was obtained. The subjects were strictly advised not to start using any antioxidants, hormones, or medications that affect the CNS. In the case of any previous use of medication with CNS effects or antioxidants, the washout time was a minimum of three months. For three weeks before and one week after the sleep studies, the participants were obliged to keep a sleep diary in order to ensure a regular sleep–wake schedule. The study was approved by the Ethical Committees of Turku University Central Hospital and the University of Helsinki.

Nocturnal polysomnography (PSG) was performed over four consecutive nights from 23:00 to 07:00. The first night served as an adaptation night. On the following evening, the participants returned to the laboratory for the baseline recording. The sleep deprivation period began at the conclusion of the baseline night (07:00) and extended to the start of the fourth night (23:00), thus lasting 40 h. The fourth night was a recovery night, after which the participants were free to leave the sleep laboratory. When allowed to sleep, participants spent the time between 23:00 and 07:00 in bed in a dark room, where only red light was used when needed. During sleep deprivation, the participants were kept awake by the study staff. Ambulatory PSG recordings were carried out during the sleep deprivation period. For serum follicle stimulating hormone (FSH) and estradiol (E2) measurements, a blood sample was drawn in the morning following the adaptation night to ensure appropriate use of HT. Blood pressure was measured every morning while supine, except on the third morning during the sleep deprivation. The Sleep Research Unit provided pre-arranged, nutritionally similar meals served at the same specific times for all participants during the study.

In all women, the PSG recordings comprised continuous monitoring of two electroencephalograms (EEG, C3/A2, C4/A1), two electro-oculograms (EOG), a mandibular electromyogram (EMG) and an ECG with a sampling frequency of 200 Hz. The postmenopausal women had two additional EEG channels (O1/A2, O2/A1) (Embla, Medcare Flaga hf. Medical Devices, Reykjavik, Iceland). A

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finger pulse oximeter was used in postmenopusal women to detect possible desaturations. All recordings were visually double-scored in 30 s epochs off-line by two experienced scorers (N.K. and P.P.-K.) according to the Rechtschaffen and Kales criteria [48] valid during the data collection (years 2001–2004). Five sleep stages (stage 1 (S1), stage 2 (S2), stages 3 and 4 (S3 and S4, SWS) and REM sleep), as well as wake time after sleep onset, were classified and expressed as percentages of total time analysed (from lights off to lights on). In addition, the number of sleep stage changes, arousals, and awakenings per hour of sleep (referred to as stage changes, awakening index and arousal index) were calculated to assess sleep fragmentation.

Heart rate variability was assessed from the overnight ECG samples from 23:00 to 07:00 during both the baseline and recovery nights using the WinCPRS® software (Absolute Aliens Ltd, Turku, Finland). All data were manually checked for artefacts and ectopic beats, and R-R detection was corrected when needed. Single, sparse ectopic beats were not corrected, but where large amounts of ectopic beats were observed, the data were discarded from the final analysis. Because of the different aspects of cardiac autonomic control addressed by linear and nonlinear approaches, both linear time and frequency domain variables and nonlinear variables were calculated.

In time domain, the standard deviation of the R-R interval (SDNN), the root mean square of successive R-R interval differences (RMSSD) and the percentage of the successive R-R intervals with over 50 ms difference in duration (pNN50) were measured [1]. The SDNN is usually considered to be a measure of overall HRV, while RMSSD and pNN50 are strongly intercorrelated measures of the vagally modulated beat-to-beat variation in heart rate [1].

In frequency domain, HRV was assessed using power spectral analysis based on the Fast Fourier transformation algorithm. Trends were removed from the signal by subtracting the regression line and the signal was then resampled with a sampling frequency of 4 Hz, using linear interpolation, and windowed using the Hanning window function, according to software standard. The Fast Fourier transformation (FFT) analysed spectra were subjected to triangular smoothing, with a range of 0.01 Hz. The frequency bands calculated were: total power 0–0.50 Hz, very low frequency (VLF) power 0.003–0.04 Hz, low frequency (LF) power 0.04–0.15 Hz, and high frequency (HF) power 0.15–0.40 Hz [1]. The VLF and HF bands are mostly products of vagally transmitted oscillations, but the LF band has a high sympathetic input. Therefore, the LF/HF ratio was calculated to estimate sympathovagal balance [1].

Nonlinear variables calculated were the Spectral Power Law (SPL) slope, the short-term and long-term R-R interval variability coefficients SD1 and SD2 from Poincaré plots, and the short and intermediate-term scaling exponents $\alpha1$ (4–11 beats) and $\alpha2$ (>11 beats) using detrended fluctuation analysis [49–51]. The SPL slope, also known as β, is the exponent of the observed $1/f^{\beta}$ decay of R-R interval spectral power in the logarithmic scale, and it is used to measure the nonlinear complexity of heart rate [52]. The Poincaré plot method is a geometric method that provides a beat-to-beat visual and quantitative analysis of R-R intervals by plotting each R-R interval of a sinus beat as a function of the previous one [50]. Detrended fluctuation analysis characterises fluctuations on scales of multiple lengths. Its scaling exponents $\alpha1$ and $\alpha2$ quantify the self-similarity in the amount of fluctuation in heart rate over short and long periods of time, respectively [49]. The Poincaré plot statistics and fractal dimension correlate with frequency domain HF power variability, which indicates that they are vagally modulated [3].

2.1. Statistical analysis

Statistical analyses were made using the IBM SPSS® version 22.0 software. Data were first tested for normality and observed to be highly skewed, and accordingly, differences between groups in HRV and sleep variables were analysed both at baseline and at recovery using the independent samples Kruskal–Wallis test. Post-hoc analyses were made using the Mann–Whitney *U*-test with Bonferroni correction. A similar analysis was applied to the sleep deprivation effect. The effect of sleep deprivation was then analysed separately in all groups using the Wilcoxon signed rank test. Heart rate variables were compared with sleep parameters at baseline and at recovery using the Spearman correlation coefficients. This analysis was also applied to the sleep-deprivation-induced change in the parameters. All results are mean (SEM) and p-values of <0.05 were considered to be significant.

3. Results

In the off-HT-group, three women were excluded from the data analysis due to frequent sinus pauses and arrhythmias. Therefore, the final groups consisted of 11 young women aged 23.1 (0.5) years and with a BMI of 23.1 (0.9) kg/m^2; 10 on-HT women aged 64.1 (1.6) years and with a BMI of 24.2 (0.6) kg/m^2; and seven off-HT women aged 64.6 (1.7) years and with a BMI of 26.1 (0.9) kg/m^2. According to the sleep diaries filled in by women before and after sleep-study nights, the sleep–wake rhythms were regular in all participants, bedtimes varied between 22:00 and 00:00 and get-up times were between 06:00 and 09:00.

The median ODI4 in the on-HT group was 3.4/h (range 0–52.2/h) (at baseline: median 3.4/h [range 0–52.2/h], at recovery: median 3.3/h [range 0.5–45/h]), with data lacking from one woman. In the on-HT group, two women had an ODI4 between 5 and 15/h, and one woman had an ODI4 over 30/h both at baseline and at recovery. One woman with an ODI4 of 4.6/h at baseline had an ODI4 of 10.1/h at recovery. In the off-HT group, median ODI4 was 2.5/h (range 0.8–10.1/h) (at baseline: median 3.8/h [range 1.0–10.1/h], at recovery: median 2.5/h [range 0.8–8.9/h]). Two women in the off-HT group had an ODI4 between 5 and 15/h both at baseline and at recovery.

The HRV variables differed between groups both at baseline and at recovery. At baseline, the young women had higher values of SDNN, RMSSD, pNN50, total power, VLF, LF, and HF powers, SD1 and SD2 than on-HT women, and higher pNN50, total, VLF and LF powers, and SD2 than off-HT women. The only differences between the postmenopausal groups at baseline were higher SDNN and SPL slopes in the off-HT women than the on-HT women (Table 1). At recovery, young women had higher values of SDNN, RMSSD, pNN50, total power, VLF, LF, and HF powers, SD1 and SD2, and lower $\alpha1$ than on-HT women, and higher pNN50 and VLF and LF powers and a lower SPL slope than off-HT women. The off-HT women had lower $\alpha1$ than on-HT women at recovery (Table 1, Fig. 1).

Compared with baseline, at recovery, total power ($p = 0.026$), VLF power ($p = 0.004$), LF power ($p = 0.033$), and LF/HF ratio ($p = 0.033$) decreased in the young women, but no changes were seen in the time domain and nonlinear indexes. In the on-HT women, mean heart rate increased ($p = 0.005$), but no other changes were found. In the off-HT group, pNN50 ($p = 0.034$), LF power ($p = 0.018$) and HF power ($p = 0.028$) decreased, and $\alpha2$ ($p = 0.043$) increased at recovery compared with baseline (Fig. 2). When the sleep deprivation effect was compared between groups, a group and sleep deprivation interaction was seen in total power ($p = 0.022$), VLF power ($p = 0.002$) and LF power ($p = 0.013$). Post-hoc analysis showed an increasing trend in total, VLF, and LF power in the on-HT women after sleep deprivation. Therefore, a group and sleep deprivation interaction effect was seen in these variables in on-HT women, as compared with young women who showed a decrease in the same variables ($p = 0.027$, $p = 0.006$ and $p = 0.042$, respectively).

The changes in sleep variables have been previously reported [53]. At recovery, the amount of wake state as well as the awakening and arousal indexes decreased, whereas the amount of SWS increased,

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Table 1
Heart rate variability indexes during baseline and recovery nights in participant groups.

	Young women			On-HT postmenopausal women			Off-HT postmenopausal women		
	Baseline	Recovery	Mean change	Baseline	Recovery	Mean change	Baseline	Recovery	Mean change
Mean RRi (msec)	978 ± 38	958 ± 29	−21	949 ± 16 ¤¤	904 ± 19	−53	1001 ± 33	924 ± 49	−77
SDNN (msec)	131 ± 12	121 ± 10	−10	68 ± 3**	70 ± 5**	3	90 ± 6§	90 ± 6	0
RMSSD (msec)	93.5 ± 15.3	82.6 ± 13.5	−10.9	32.8 ± 6.2**	27.7 ± 3.7**	−6.2	58.6 ± 11.6	45.6 ± 10.9	−13.0
pNN50 (%)	37.4 ± 5.8	35.2 ± 5.2	−2.3	7.0 ± 3.4**	5.2 ± 1.9**	−2.0	8.7 ± 1.9 ¤*	5.2 ± 1.7**	−3.5
Total power (msec^2)	12,318 ± 2215 ¤	9095 ± 1571	−3223	3151 ± 262**	3443 ± 555**	336*	5578 ± 897	4410 ± 795	−1168
VLF power (msec^2)	4132 ± 551 ¤¤	3003 ± 351	−1128	1282 ± 116**	1394 ± 232*	113**	1695 ± 335*	1284 ± 223*	−411
LF power (msec^2)	2663 ± 651 ¤	1830 ± 415	−833	448 ± 66**	473 ± 92**	19*	535 ± 167 ¤**	300 ± 95**	−235
HF power (msec^2)	2402 ± 681	2068 ± 617	−333	279 ± 89**	203 ± 51**	−82	665 ± 265 ¤	331 ± 155*	−335
LF/HF ratio (%)	160 ± 28 ¤	127 ± 23	−32	279 ± 89	302 ± 45**	25	133 ± 44	137 ± 31§	4
SPL slope	0.764 ± 0.059	0.730 ± 0.071	−0.034	0.942 ± 0.065	0.943 ± 0.067	−0.007	1.191 ± 0.079**§	1.284 ± 0.156**	0.093
Poincaré SD1	69.8 ± 11.2	58.5 ± 9.5	−11.3	23.3 ± 4.3**	19.5 ± 2.6**	−4.6	41.0 ± 8.4	32.3 ± 7.6	−8.7
Poincaré SD2	176.5 ± 16.1	159.1 ± 11.8	−17.4	92.9 ± 4.8**	96.7 ± 6.9**	4.8	118.9 ± 7.9	121.6 ± 8.9	2.7
α1	0.922 ± 0.060	0.876 ± 0.056	−0.045	1.145 ± 0.086	1.178 ± 0.046**	0.052	0.813 ± 0.073	0.822 ± 0.070§§	0.009
α2	0.955 ± 0.022	0.974 ± 0.024	0.020	0.957 ± 0.023	0.993 ± 0.030	0.048	0.990 ± 0.051 ¤	1.052 ± 0.042	0.062

α1, detrended fluctuation analysis short-term scaling exponent; α2, detrended fluctuation analysis intermediate-term scaling exponent; HF power, high frequency power of HRV; HT, hormone therapy; LF power, low frequency power of HRV; Poincaré SD1, Poincaré plot short term variability coefficient; Poincaré SD2, Poincaré plot long term variability coefficient; pNN50, percentage of R-R intervals with over 50 ms difference in duration; RMSSD, root mean square of successive R-R intervals; RRi, R-R peak interval; SDNN, standard deviation of R-R intervals; SPL slope, spectral power law slope; VLF power, very low frequency power of heart rate variability (HRV).

* $p < 0.05$, **$p < 0.01$ compared with young women.

¤ $p < 0.05$, ¤¤$p < 0.01$ baseline vs recovery.

§ $p < 0.05$, §§$p < 0.01$ compared with on-HT postmenopausal women.

compared with baseline. Group differences were seen in the amounts of S1, SWS, REM and wake state, and in the awakening index both at baseline and at recovery. The amount of sleep stage changes decreased from baseline to recovery only in on-HT women. This change differed from that of young women ($p = 0.038$) (Table 2).

When analyzing the connections between sleep variables and HRV indexes at baseline, time spent awake was negatively correlated with RMSSD, pNN50, VLF and LF powers, and SD1, and positively correlated with SPL slope. Time spent in S1 sleep at baseline was negatively correlated with SDNN, RMSSD, pNN50, total power, VLF, LF and HF powers, and SD1. Time spent in S2 sleep at baseline was negatively correlated with α2. Time spent in SWS at baseline was negatively correlated with SPL slope, and positively correlated with SDNN, RMSSD, pNN50, total, VLF, and HF powers, and SD1. Time spent in SWS at baseline correlated positively with LF power. Time spent in REM sleep at baseline was negatively correlated with mean and maximum heart rates.

At recovery, time spent awake was negatively correlated with SDNN, RMSSD, pNN50, VLF, LF and HF powers, SD1, and SD2, and positively correlated with SPL slope and LF/HF ratio. Time spent in S1 sleep at recovery was negatively correlated with SDNN, RMSSD, pNN50, total power, HF power, SD1, and SD2, and positively correlated with LF/HF ratio and minimum heart rate. Time spent in S2 sleep at recovery was negatively correlated with α2. Time spent in SWS at recovery was negatively correlated with minimum heart rate, LF/HF ratio, and α1,and positively correlated with SDNN, RMSSD, pNN50, total, VLF, and HF powers, SD1, and SD2. Time spent in REM sleep at recovery was negatively correlated with SDNN, pNN50, total and LF powers, and SD2, and positively correlated with minimum heart rate (Table 3).

At baseline, awakening index was negatively correlated with SDNN, RMSSD, pNN50, total, VLF, LF and HF powers, SD1 and SD2. Sleep stage changes at baseline were negatively correlated with SDNN, total and HF powers, and SD2, and positively correlated with LF/HF ratio. Arousal index was negatively correlated with α2 at baseline. At recovery, awakening index was negatively correlated with SDNN, RMSSD, pNN50, total, VLF, LF and HF powers, SD1, and SD2 and positively correlated with LF/HF ratio and α1 (Table 3).

When the change in sleep variables between baseline and recovery nights was compared with changes in HRV, it was observed that the decrease in the amount of wake was associated with a decrease in maximum heart rate ($r = 0.564$, $p = 0.002$), and the decrease in the amount of S1 sleep was associated with a decrease in LF/HF ratio ($r = 0.404$, $p = 0.037$). An increase in S2 sleep was associated with a decrease in α2 ($r = -382$, $p = 0.049$). The decrease in awakening index was associated with a decrease in maximum heart rate

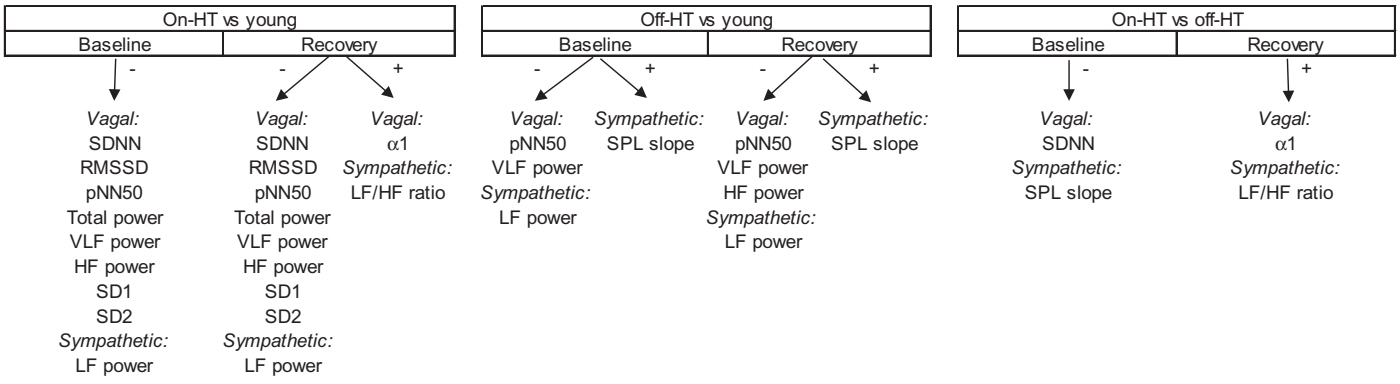

Fig. 1. Differences in between group heart rate variability indexes. Minus sign indicates lower values, and plus sign higher values in the first mentioned group. Vagal, indexes under mainly vagal influence; sympathetic, indexes under mainly sympathetic influence.

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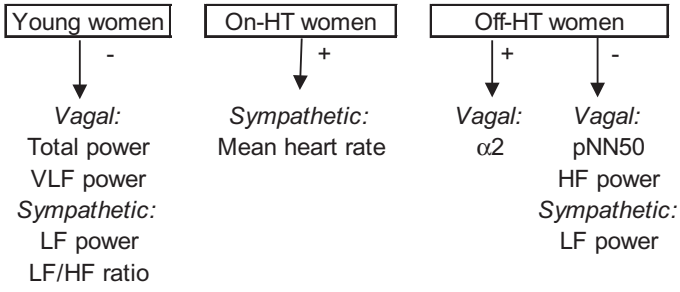

Fig. 2. Changes in heart rate variability indexes from baseline to recovery. Minus sign indicates a decrease, plus sign an increase at recovery. Vagal, indexes under mainly vagal influence; sympathetic, indexes under mainly sympathetic influence.

($r = 0.445$, $p = 0.020$). No other correlations between sleep variable changes and HRV changes were found.

To control for the possible effect of the on-HT woman with severe sleep-disordered breathing (ODI4 >45/h) in the results, a reanalysis of all data was performed with the omission of this woman from the database. When comparing the results with those presented here, they remained essentially the same.

4. Discussion

The present study was the first to evaluate the effect of age and HT on nocturnal HRV after acute total sleep deprivation in women. The results showed a slight decrease in frequency domain HRV and LF/HF ratio in young women during a recovery night. This indicates that although a stress effect was evident, sympathetic output was still overruled by vagal output. These findings are in line with previous findings in young men [23]. In postmenopausal women, who were not using HT, a decrease in frequency domain HRV was also seen at recovery, but without LF/HF ratio changes, indicating no change in sympathovagal balance. In contrast, in postmenopausal women using HT, the only change was an increase in mean heart rate. Further, when compared with women without HT, women using HT had slightly higher sympathetic dominance and a tendency for decreased vagal output. In addition, both linear and nonlinear HRV parameters decreased with increasing age at baseline and during recovery. Vagal tone was higher in women with more SWS and less S1 sleep, wake, and awakenings both at baseline and at recovery. However, the consolidation of sleep with less stage changes, awakenings and arousals, and the shift in sleep stages favouring deep sleep over light sleep and wake state at recovery had only minor, albeit logical effects on HRV, indicating a slight increase in vagal tone.

When the effect of age on HRV is considered, the present study confirmed the results of previous population-based studies, where both time and frequency domain HRV indexes, baroreflex sensitivity and entropy have been shown to decrease, and LF/HF ratio, α1 and α2 to increase with age [54–56]. In the present study, the focus was on aging women, particularly on the drastic change in their physiology elicited by the hormonal changes during menopause. This change is reflected in similar HRV in postmenopausal women and same-age men [57], while in premenopausal middle-aged women, heart rate entropy and HF power are higher, and α1, LF power and LF/HF ratio lower than in men of the same age [37,54,57]. The magnitude of age-related change in HRV indexes observed in previous studies is similar to that observed in this population, although the exact values differ between studies [54–56]. This, however, is mostly explained by the fact that the reference studies had gender-mixed populations, while the present population was completely female.

The women using HT had the lowest values of vagally modulated linear and nonlinear HRV and the strongest sympathetic dominance of all women enrolled in the present study. This possibly also influenced the lack of effect of sleep deprivation on their HRV. Of importance here is that the postmenopausal population averaged an age of approximately 64 years, being at least 10 years postmenopausal. They also had a history of HT self-chosen use with 2 mg of estradiol hemihydrate, which is considered to be a high dose according to present recommendations. This implies a higher risk for cardiovascular disease and stroke in the study group [24,58,59]. In studies conducted on HT in younger perimenopausal or recently postmenopausal women, an increase in the linear indexes of HRV and a decrease in LF/HF ratio have been observed [37–42]. However, in studies on older postmenopausal populations, the effect of HT on linear HRV has been neutral [43,44], or a decrease in both vagally modulated linear HRV indexes and nonlinear complexity of heart rate has been observed [45–47]. Previous study results seem to be unconnected to the application route of HT, the addition of progestagens or the dose of oestrogen used. Evidence from the WHI and the HERS re-evaluations [31,32] and other recent studies [33–36] have suggested that both oestrogen only HT and combined oestrogen–progestagen HT initiated early after menopause decreases cardiovascular morbidity and mortality in the long run, whereas in older populations, the effect is the opposite [31]. It has been shown that decreased vagal tone, as measured by HRV, is

Table 2
Sleep variables during baseline and recovery nights in participant groups.

	S1	S2	SWS	REM^	Wake	Stage changes	Awakening Index	Arousal Index
Young ($n = 11$)								
Baseline	6.4% ± 0.8% §§	43.7% ± 1.2%	22.1% ± 1.0% §§	21.0% ± 1.3%	3.7% ± 1.6% §	18.0 ± 1.2	0.76 ± 0.19 §§	9.5 ± 0.9 §§
Recovery	3.3% ± 0.6%	43.5% ± 1.2%	33.5% ± 1.6%	18.5% ± 1.1%	0.2% ± 0.1%	17.8 ± 1.1	0.15 ± 0.04	7.4 ± 0.9
On-HT ($n = 10$)								
Baseline	11.0% ± 1.1%* §	47.3% ± 2.3%	8.1% ± 1.4%** §§	17.4% ± 1.2%	12.9% ± 2.2%* §	22.7 ± 1.7 § ¤	3.37 ± 0.59** §§	11.4 ± 1.6 §
Recovery	6.5% ± 1.0%*	49.9% ± 2.7%	16.9% ± 2.3%**	20.9% ± 1.7%	4.1% ± 0.7%**	18.1 ± 1.5	1.56 ± 0.20**	6.6 ± 1.1
Off-HT ($n = 7$)								
Baseline	9.7% ± 1.3% §	43.7% ± 3.0%	12.7% ± 2.5%* §	16.2% ± 0.9%	14.7% ± 1.9%**	19.5 ± 1.9	2.18 ± 0.26*	10.5 ± 1.3 §
Recovery	5.3% ± 0.7%	39.9% ± 2.5%	25.0% ± 3.0%	19.9% ± 2.3%	8.0% ± 2.1%**	18.1 ± 1.3	1.31 ± 0.18**	6.3 ± 0.7

Arousal index, arousals per hour of sleep; awakening index, awakenings per hour of sleep; REM, REM sleep; S1, stage 1 non-REM sleep; S2, stage 2 non-REM sleep; stage changes, sleep stage changes per hour of sleep; SWS, slow wave sleep.

* $p < 0.05$, ** $p < 0.01$ vs young women.

^ In Kruskal–Wallis test for independent samples, $p = 0.037$ for between-group differences in percentage change at baseline, post-hoc differences not significant.

¤ Significant group difference for the change between baseline and recovery as compared with young women at $p = 0.038$.

§ $p < 0.05$, §§$p < 0.01$ recovery vs baseline.

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Table 3
Correlation between heart rate variability indexes and sleep variables ($n = 27$) (sleep stage information lacking from one on-HT woman).

		S1	S2	SWS	REM	Wake	Stage changes	Awakening Index	Arousal Index
Minimum RRi	Baseline	–	–	–	0.484*	–	–	–	–
	Recovery	–	–	–	–	–	–	–	–
Maximum RRi	Baseline	–	–	–	–	–	–	–	–
	Recovery	−0.426*	–	0.390*	–	−0.419*	–	–	–
Mean RRi	Baseline	–	–	–	0.435*	–	–	–	–
	Recovery	–	–	–	–	–	–	–	–
SDNN	Baseline	−0.588**	–	0.471*	–	–	−0.474*	−0.647**	–
	Recovery	−0.419*	–	0.590**	−0.537**	−0.514**	–	−0.623**	–
RMSSD	Baseline	−0.507**	–	0.481*	–	−0.391*	–	−0.567**	–
	Recovery	−0.465*	–	0.559**	–	−0.607**	–	−0.659**	–
pNN50	Baseline	−0.536**	–	0.610**	–	−0.560**	–	−0.695**	–
	Recovery	−0.481*	–	0.569**	−0.400*	−0.787**	–	−0.767**	–
Total power	Baseline	−0.482*	–	0.454*	–	−0.382*	−0.388*	−0.606**	–
	Recovery	−0.387*	–	0.496**	−0.398*	−0.486*	–	−0.603**	–
VLF power	Baseline	−0.423*	–	0.460*	–	−0.426*	–	−0.591**	–
	Recovery	–	–	0.493**	–	−0.608**	–	−0.603**	–
LF power	Baseline	−0.443*	–	0.534**	–	−0.473**	–	−0.596**	–
	Recovery	–	–	–	−0.428*	−0.680**	–	−0.576**	–
HF power	Baseline	−0.540**	–	0.483*	–	–	−0.426*	−0.620**	–
	Recovery	−0.460*	–	0.563**	–	−0.728**	–	−0.768**	–
LF/HF ratio	Baseline	0.416*	–	–	–	–	0.405*	–	–
	Recovery	0.513**	–	−0.615**	–	0.446*	–	0.672**	–
SPL slope	Baseline	–	–	−0.440*	–	0.523**	–	–	–
	Recovery	–	–	–	–	0.564**	–	–	–
Poincaré SD1	Baseline	−0.505**	–	0.476*	–	−0.390*	–	−0.559**	–
	Recovery	−0.460*	–	0.559**	–	−0.607**	–	−0.662**	–
Poincaré SD2	Baseline	−0.565**	–	0.479*	–	–	−0.424*	−0.632**	–
	Recovery	−0.426*	–	0.620**	−0.548**	−0.543**	–	−0.631**	–
α1	Baseline	–	–	–	–	–	–	–	–
	Recovery	–	–	−0.593**	–	–	–	0.495**	–
α2	Baseline	–	−0.567**	–	–	–	–	–	−0.436*
	Recovery	–	−0.451*	–	–	–	–	–	–

α1, detrended fluctuation analysis short-term scaling exponent; α2, detrended fluctuation analysis intermediate-term scaling exponent; arousal index, arousals per hour of sleep; awakening index, awakenings per hour of sleep; HF power, high frequency power of HRV; LF power, low frequency power of HRV; maximum RRi, maximum R-R peak interval (reverse of maximum heart rate); mean RRi, mean R-R peak interval (reverse of mean heart rate); minimum RRi, minimum R-R peak interval (reverse of maximum heart rate); pNN50, percentage of R-R intervals with over 50 ms difference in duration; Poincaré SD1, Poincaré plot short term variability coefficient; Poincaré SD2, Poincaré plot long term variability coefficient; REM, REM sleep; RMSSD, root mean square of successive R-R intervals; RRi, R-R peak interval; S1, stage 1 non-REM sleep; S2, stage 2 non-REM sleep; SDNN, standard deviation of R-R intervals; SPL slope, spectral power law slope; stage changes, sleep stage changes per hour of sleep; SWS, slow wave sleep; VLF power, very low frequency power of heart rate variability (HRV).

* $p < 0.05$, **$p < 0.01$.

associated with increased cardiovascular mortality [5–8] and in a recent subset analysis of the WHI, ECG parameters linked to decreased vagal function were shown to associate with increased overall mortality [9]. The results of this study therefore support the evidence that HRV may indeed be a factor in the cardiovascular mortality increasing effect of HT in women well past their menopause.

The awake state and light sleep have previously been shown to be associated with higher sympathetic output than other sleep stages, while vagal influence predominates in SWS [10–13,60]. This relationship does not change during menopause [61]. In the present study, the subject-by-subject correlation between HRV indexes and sleep stage changes both at baseline and at recovery was in line with these previous findings in both young and older women. Additionally, it was observed that a higher amount of awakenings was correlated to lower vagal and higher sympathetic output. A similar observation about the effect of awakenings has been achieved by studying patients with sleep-related breathing disorders and periodic limb movements during sleep, where sleep fragmentation due to these illnesses resulted in higher LF/HF ratios [44]. This indicates high sympathetic tone leading to high cardiovascular risk [5–8].

In the present study, the changes in sleep variables between baseline and recovery nights showed a rebound effect with deeper and more consolidated sleep in all study groups at recovery [53], a finding first documented several decades ago [62]. However, no concomitant increase in vagally mediated HRV was seen, probably indicating that the body has not yet recovered from the burden of sleep deprivation. Instead, a decrease in several, both vagally and sympathetically modulated, frequency domain variables was observed in young and in off-HT women. In young women only, a small decrease in sympathetic dominance was observed at recovery, but its clinical significance remains debatable. In the older groups, no significant differences in sympathovagal balance could be observed. Furthermore, correlations between the effects of recovery sleep on sleep variables and HRV were scarce and random, supporting the above. Previously, sleep deprivation effects on HRV have been evaluated either in all-male or gender-mixed young populations with conflicting results. In a study in 12 male and 12 female volunteers aged 27–45 years, acute sleep deprivation of 24 h did not influence HRV [18] measured in the awake state. Another study in six men and six women aged 18–30 years showed an increased sympathetic tone during the day after sleep deprivation, but increased respiratory sinus arrhythmia as a marker of increased parasympathetic tone during the subsequent recovery night [19]. In a population of 10 young men aged 23 ± 1 years, an increased HF power after 24 h of sleep deprivation was found [20], and one study in 17 men and three women aged 26 ± 3 years [21] reported an increase in both HF and LF variability. Recent studies with 24–60 h of continuous sleep deprivation in male-dominant populations with mean ages between 25 and 27 years showed increased LF power or LF/HF ratio and decreased HF power, pNN50 and RMSSD while awake during or after prolonged sleep deprivation, indicating

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increased sympathetic dominance [14–17], while a decrease in sympathovagal balance was observed late in the evening or during recovery sleep in two studies on mostly male shift-working physicians under 49 years of age (24,25). The present group-wise results did not markedly differ from these findings. However, this was the first study to include female subjects over 60 years, thus increasing the potential to generalise the observations to a larger population.

The present study has limitations concerning the sleep stage and saturation analyses. The data were collected in two different centres, and for the young women, only C3/C4 electrodes were used for collecting EEG, and oximetry was lacking due to limited resources at the study site. The lack of posterior scalp EEG limits the assessment of posterior alpha activity especially, and therefore the differentiation between wake and drowsiness [63]. However, as the shortage of electrodes applied only to the young women, who had very clear-cut EEG patterns, this unlikely formed an error in the sleep stage analysis. Since the sleep data were collected and analysed between the years 2001 and 2004, the sleep stage analysis was performed using Rechtschaffen and Kales criteria [48], instead of the American Academy of Sleep Medicine (AASM) criteria first established in 2007 [64]. This may have caused bias, leading to the overestimation of S2/N2 sleep and underestimation of both S1/N1 sleep and SWS in the data [65]. As the bias was constant and applied to both high and low vagal input states in the context of sleep, for the purposes of this study it was chosen not to rescore the sleep data. As for the lack of oximetry data in young women, the likelihood for sleep-disordered breathing was considered to be small, as their mean age was below 25 years and their BMI was within normal limits. The one postmenopausal woman with high ODI4, indicating severe sleep-disordered breathing, was another concern, as sleep-related breathing disorders affect sympathovagal balance [64]. As the reanalysis of all data with the omission of this woman showed no change in the results, to increase statistical power it was decided to include her data in the report.

A further limitation concerns the approach used to calculate HRV. In the present study, it was decided to select the whole recorded segment of ECG, from lights off to lights on, instead of reporting HRV in all sleep stages separately. The later approach would have been problematic due to technical limitations of the mathematical paradigms used to calculate HRV indexes. The Poincaré plot variables and $\alpha 2$ require lengthy periods of time for reliability [1,66] (eg, SDNN needs time sections of exactly identical lengths to be comparable between epochs) [1]. The epochs of wake and stage 1 sleep were rather short in many women, and therefore could not have been compared with epochs of other sleep stages. Based on this rationale, an overnight approach was considered to be more appropriate for the present study.

The implications of the present study lie in the fact that while an acute, highly demanding event (eg, acute sleep deprivation) is harmful for a young body, it is even more harmful for an aging body. Further, use of HT in late menopause does not seem to give protection against these changes. The changes in HRV similar to those seen in the present study after total sleep deprivation are known to be associated with an increased risk of cardiovascular morbidity and mortality, and overall mortality [5–8]. Previous literature has confirmed an increased risk on acute myocardial infarction in people sleeping 5 h or less per night, in both men [67] and women [68]. Furthermore, both increased risk of cardiovascular and overall mortality is significantly increased in short-time sleepers [69–71], especially in women and elderly people [72,73]. Therefore, it may be assumed that, although the context of long-standing partial sleep loss differs from the present research paradigm, the changes in autonomic nervous system function seen in the study may at least, in part, explain the connection with the morbidity and mortality changes associated with sleep loss seen in observational studies.

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.10.012.

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